Anatomic Pathology Selected Abstracts, 2/13

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BRCA1 and BRCA2 mutations, TP53 abnormalities, and immune cell infiltrates in ovarian carcinoma

The authors characterized *BRCA1* and *BRCA2* status (mutation/methylation) in a consecutive series of cases of ovarian carcinoma to identify differences in clinicopathological features, molecular characteristics, and outcome between the pelvic high-grade serous cancers with germline or somatic mutations in *BRCA1* or *BRCA2*, methylation of *BRCA1*, and normal *BRCA1* or *BRCA2*. A total of 131 women were identified prospectively, all of whom were undergoing surgical staging and agreed to germline testing for *BRCA1* and *BRCA2* mutations. Histopathology, germline and somatic *BRCA1* or *BRCA2* mutations, *BRCA1* methylation, and *BRCA1* and *BRCA2* mRNA expression levels distinguished four subgroups. In all, 103 cases were high-grade serous carcinoma, and of these, 31 (30 percent) had germline or somatic *BRCA1* or *BRCA2* mutations (20 percent *BRCA1* and 10 percent *BRCA2*; group one); 21 (20 percent) had methylation of *BRCA1* (group two); and 51 (50 percent) had no BRCA loss (group three). Group four consisted of 28 cases of non-high–grade serous carcinoma, none of which had BRCA loss. *BRCA1* and *BRCA2* mRNA expression levels correlated with designated group (P=.0008). Among high-grade serous carcinomas, there were no differences between groups one through three with respect to stage, ascites, CA125 level, platinum sensitivity, cytoreduction rate, neoadjuvant chemotherapy, or survival. Tumors with *BRCA1* or *BRCA2* mutations had increased immune infiltrates (CD20 and TIA-1) compared with high-grade serous tumors without mutations (P=.034; .027). *TP53* expression differed between groups (P<.0001), with abnormal *TP53* expression in 49 of 50 tumors from groups one and two. Wild-type *TP53* expression was associated with worse outcome in high-grade serous tumors (P<.001). BRCA loss (mutation/methylation) is a common event in pelvic high-grade serous tumors (50 percent). *TP53* abnormalities and increased immune cell infiltrates are significantly more common in high-grade serous tumors with germline and somatic mutations in *BRCA1* or *BRCA2* compared with tumors lacking BRCA abnormalities.

McAlpine JN, Porter H, Köbel M, et al. *BRCA1* and *BRCA2* mutations correlate with *TP53* abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. *Mod Pathol*. 2012;25:740–750.
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Significance of loss of ARID1A/BAF250a expression in endometriosis

Mutations of the tumor-suppressor gene *ARID1A* result in the loss of protein expression of the BRG-associated factor 250a (BAF250a), a large subunit of transcription-regulating human SWI/SNF complexes that play an important role in controlling cell proliferation and in tumor suppression. *ARID1A* mutations are particularly frequent in endometriosis-associated ovarian clear cell and endometrioid carcinomas and were recently described as a possible key mechanism and early step in the transformation of endometriosis into cancer. The authors conducted a study in which they examined the immunohistochemical expression pattern of BAF250a in a tissue microarray including 74 endometriosis and 30 endometrium samples. Ovarian cancer samples (n=136) served as a control. Epithelial BAF250a expression was assessable in 90 of 104 (87 percent) and stromal BAF250a expression in 95 of 104 (91 percent) of the endometriosis and endometrium cases due to lack of adequate tissue in some spots. Complete lack of BAF250a expression was observed in three endometriomas (n=3 of 20; 15 percent) and one deep-infiltrating endometriosis sample (n=1 of 22; five percent) but in none of the peritoneal endometriosis (n=0

of 16) and eutopic endometrium samples (n=0 of 30). A comparison of the mean immunoreactivity scores revealed a significantly lower expression rate of BAF250a in endometriomas compared with normal endometrium (*P*<.0005), as well as in peritoneal (*P*=.003) and deep-infiltrating endometriosis (*P*=.02). The authors' data demonstrate that a complete loss of BAF250a expression is observable in some endometriotic lesions, especially in endometriosis. In addition, the authors reported that a partial loss of BAF250a expression is occurring in the form of cell clusters, indicating a clonal loss of BAF250a expression in these cells. The loss of expression of the tumor-suppressor protein BAF250a in some endometriomas possibly indicates a risk of malignant transformation in these cases, which could be important in determining individual treatment strategies. However, its role and value as a prognostic parameter in endometriosis needs to be studied further.

Samartzis EP, Samartzis N, Noske A, et al. Loss of ARID1A/BAF250a-expression in endometriosis: a biomarker for risk of carcinogenic transformation? *Mod Pathol*. 2012;25:885–892.
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Relationship between pathologic complete response and prognosis after chemotherapy in breast cancer subtypes

The exact definition of pathologic complete response and its prognostic impact on survival in intrinsic breast cancer subtypes is uncertain. The authors conducted a study in which they analyzed tumor response at surgery and its association with long-term outcome for 6,377 patients with primary breast cancer who were receiving neoadjuvant anthracycline-taxane–based chemotherapy in seven randomized trials. The authors found that disease-free survival was significantly superior in patients with no invasive and no in situ residuals in breast or nodes (n=955) compared with patients with residual ductal carcinoma in situ only (n=309), no invasive residuals in breast but involved nodes (n=186), only focal-invasive disease in the breast (n=478), and gross invasive residual disease (n=4,449; *P*<.001). Hazard ratios for disease-free survival comparing patients with or without pathologic complete response (pCR) were lowest when defined as no invasive and no in situ residuals (0.446) and increased when the definition included in situ residuals (0.523), no invasive breast residuals but involved nodes (0.623), and focal-invasive disease (0.727). Pathologic complete response was associated with improved disease-free survival in tumors that were luminal B/human epidermal growth factor receptor 2 (HER2) negative (*P*=.005), HER2 positive/nonluminal (*P*<.001), and triple negative (*P*<.001) but not in luminal A (*P*=.39) or luminal B/HER2-positive (*P*=.45) breast cancer. Pathologic complete response in HER2-positive (nonluminal) and triple-negative tumors was associated with excellent prognosis. The authors concluded that pCR defined as no invasive and no in situ residuals in breast and nodes can best discriminate between patients with favorable and unfavorable outcomes. Patients with noninvasive or focal-invasive residuals or involved lymph nodes should not be considered as having achieved pCR. Pathologic complete response is a suitable surrogate endpoint for patients with luminal B/HER2-negative, HER2-positive (nonluminal), and triple-negative disease but not for those with luminal B/HER2-positive or luminal A tumors.

von Minckwitz G, Untch M, Blohmer JU, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol.* 2012;30:1796–1804.
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Determining HER2 status on breast core-needle biopsies

Preoperative breast cancer diagnosis on core biopsies has become a standard of care in many countries. Controversy surrounds the accuracy of HER2 testing on biopsies as compared with surgical specimens, and few data exist concerning the use of emerging technologies, such as bright-field in situ hybridization, in such a setting. A French multicenter, cross-sectional, histopathological study assessed the concordance of HER2 status determined by immunohistochemistry and silver or chromogenic in situ hybridization (SISH and CISH, respectively) on core-needle biopsies with HER2 status determined by fluorescence in situ hybridization (FISH) performed on

surgical specimens. The concordance between biopsy and operative results was also assessed for each method. The authors studied 260 breast tumors from 24 centers between April 2003 and August 2009. Excellent concordance (k, 0.92–0.97) was shown between immunohistochemistry and FISH, with low discordance rates (two to four percent) and high specificity (97 to 98 percent) and sensitivity values (95 to 99 percent), with no significant difference according to the immunohistochemistry interpretation guidelines used. The correlation between SISH and CISH on biopsies and FISH on surgical samples was strong (k, 0.96 and 0.94, respectively), with no significant difference between false-negative rates or sensitivity and specificity values (two and five percent, 99 and 96 percent, and 98 and 98 percent, respectively). Whatever the evaluation technique, excellent concordance between biopsies and surgical specimens was observed (k ³ 0.97; discordance rates between one and two percent), with high sensitivity (98 to 99 percent) and specificity (98 to 100 percent). Based on these results, when FISH cannot be used, SISH or CISH, or both, could be proposed as an alternative method to determine HER2 status and to confirm ambiguous immunohistochemistry results, either for preoperative percutaneous biopsies or surgical specimens. CISH and SISH could also be used for quality controls and immunohistochemistry calibration.

Arnould L, Roger P, Macgrogan G, et al. Accuracy of HER2 status determination on breast core-needle biopsies (immunohistochemistry, FISH, CISH and SISH vs. FISH). *Mod Pathol*. 2012;25:675–682.
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Use of MiTF in differentiating cellular neurothekeoma from plexiform fibrohistiocytic tumor

The overlapping histopathologic features of cellular neurothekeoma and plexiform fibrohistiocytic tumor, when both are predominantly composed of histiocytoid cells, make it difficult to distinguish between these entities. Some have suggested that they are related. No prior study has offered a reliable immunohistochemical panel to differentiate these entities. The authors conducted a study in which they retrieved skin biopsies diagnosed in the timeframe of 2004 to 2010 as cellular neurothekeoma (CNT) and plexiform fibrohistiocytic tumor (PFHT) and obtained the accompanying pathology reports. Each case was reviewed by at least two dermatopathologists and two soft tissue pathologists to confirm the diagnosis. All cases were then evaluated for immunohistochemical expression of PAX2, NKIC3, CD10, and microphthalmia transcription factor (MiTF). The authors found that, histopathologically, the histiocytoid areas of each tumor shared similar architecture, demonstrating nests and fascicles of histiocytoid to spindled cells, with some separation of nests by collagen bands. Both CNT and PFHT were uniformly positive for NKIC3 and CD10, and both were frequently PAX2 positive. MiTF was strongly and diffusely positive in CNT and consistently negative in PFHT. The authors concluded that CNT and PFHT share many histopathologic features and immunohistochemical staining patterns. Expression of MiTF may be a reliable marker for distinguishing CNT from histiocytoid-predominant PFHT, especially in instances where only a small part of the tumor is sampled for evaluation.

Fox MD, Billings SD, Gleason BC, et al. Expression of MiTF may be helpful in differentiating cellular neurothekeoma from plexiform fibrohistiocytic tumor (histiocytoid predominant) in a partial biopsy specimen. *Am J Dermatopathol.* 2012;34(2):157–160.
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A study of genetic heterogeneity in HER2/neu testing by FISH

Amplification of the *ERBB2* oncogene encoding HER2/*neu* protein (HER2) is of predictive and prognostic importance in breast carcinoma. Fluorescence in situ hybridization (FISH) is a widely accepted method for determining HER2 amplification status. A HER2-amplified tumor is defined as having a ratio of HER2 signals to chromosome 17 centromeric probe signals (HER2/CEP17 ratio) exceeding 2.2. However, the presence of scattered cells demonstrating HER2 amplification is of unclear significance. A 2009 panel guideline defined a tumor with genetic

heterogeneity as having at least five percent but fewer than 50 percent of (non-clustered) tumor nuclei with a ratio greater than 2.2. The authors conducted a study to examine the statistical distribution of breast tumors tested by FISH for HER2 amplification after implementation of this 2009 guideline. They identified 2,522 consecutive breast carcinoma cases (2009–2011) tested for HER2 amplification. All cases were tested by FISH using a standard clinical protocol, adhering to established guidelines. For each case, data on cell counts were retrieved electronically. Each tumor was compared with a theoretical normal distribution by quantile-quantile analysis. Of 2,522 FISH tests for HER2, 1,900 (75 percent) were non-amplified, 394 (16 percent) were amplified, and 228 (nine percent) were HER2 equivocal. A total of 666 (26 percent) had genetic heterogeneity. Among these genetically heterogeneous cases, the ratio was non-amplified in 430 (64.5 percent), amplified in 24 (four percent), and equivocal in 212 (31.5 percent). The amplified subpopulation in genetically heterogeneous tumors was larger if the overall ratio was close to 2.2. However, the percentage of nuclei greater than 2.2 in a genetically heterogeneous tumor was not informative of the underlying tumor-cell distribution. The authors concluded that the proportion of HER2-amplified nuclei within a tumor does not contribute information independent of the HER2/CEP17 ratio. Reassessment of the definition of genetic heterogeneity in HER2 testing is warranted.

Chang MC, Malowany JI, Mazurkiewicz J, et al. ‘Genetic heterogeneity’ in HER2/neu testing by fluorescence in situ hybridization: a study of 2522 cases. *Mod Pathol*. 2012;25:683–688.
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